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Physicochemical stability of citral emulsions stabilized by milk proteins (lactoferrin, α-lactalbumin, β-lactoglobulin) and beet pectin

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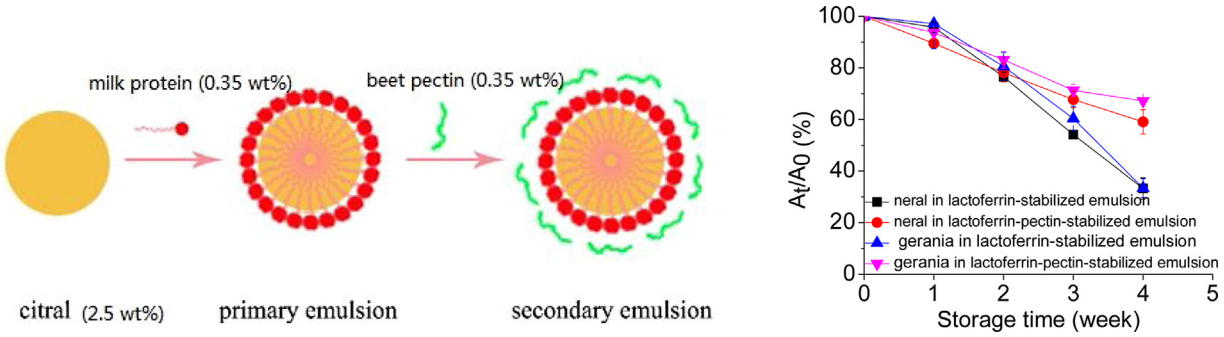
GRAPHICAL ABSTRACT



HIGHLIGHTS

- Bilayer citral emulsions were prepared with different milk proteins (lactoferrin, α-lactalbumin, β-lactoglobulin) and beet pectin (BP).
- Bilayer emulsions exhibited better physical stability against environmental stresses than monolayer emulsions.
- Bilayer emulsions could better protect neral and geranial from oxidation during the storage at 25 °C.
- Lactoferrinn-beet pectin stabilized emulsion exhibited the best physicochemical properties among all secondary emulsions.

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ABSTRACT

Citral emulsions were prepared by the layer-by-layer deposition technique based on the electrostatic interaction between positively charged milk proteins (lactoferrin, α-lactalbumin or β-lactoglobulin, at pH 3.5) and a negatively charged polysaccharide (beet pectin), which had different interfacial compositions: (i) primary emulsions (milk proteins); (ii) secondary emulsions (milk protein-beet pectin). The effects of environmental stresses (ionic strength, pH, thermal treatment) on the physicochemical stability of citral emulsions were investigated. In the absence of beet pectin, citral emulsions were highly unstable and aggregated at pH close to the pI of the proteins and NaCl concentration of 0.1–0.5 M. The droplets in milk protein-beet pectin coated emulsions were stable against the aggregation at pH range of 3.5–10 and NaCl concentration of 0.1–0.5 M. During the storage of 4 weeks, secondary emulsions exhibited better physical stability than primary ones and could protect the isomers (neral and geranial) of citral from degradation at 25 °C. In general, lactoferrinn-beet pectin stabilized emulsion exhibited the best physicochemical properties among emulsions involved in this study.

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1. Introduction

Citral (3,7-dimethyl-2, 6-octadienal), an important natural flavoring compound, is widely applied as an additive in foods [1], beverages and cosmetics for its strong lemon odour [2]. It consists of two geometrical isomers, neral and geranial, and is highly prone to acid-catalysed and oxidative degradation, leading to loss of attractive flavor and formation of off-flavors. Therefore, the incorporation of citral into foods and beverages is a major challenge for food industry because its chemical deterioration needs to be inhibited to minimize loss of product quality.

In food industry, proteins and polysaccharides are commonly used as emulsifiers to stabilize oil-in-water emulsions. Adsorption of proteins frequently takes place at liquid/liquid interfaces, the states of protein adsorption in emulsion systems play an important role in forming different emulsion-based products [3]. In general, proteins can stabilize the emulsion through electrostatic and/or steric repulsive forces, however, at the isoelectric point (pI), the net surface charge of protein molecules was zero, leading to droplets aggregation [4]. Most polysaccharides could form an extended network in the aqueous phase to stabilize emulsions. Some polysaccharides or their derivatives could adsorb at the oil–water interface, such as arabic gum, modified starch and pectin [5].

Accordingly, there is a growing interest in combining proteins and polysaccharides to form electrostatic complexes to stabilize emulsions. Combrinck et al. [6] prepared emulsions with whey protein and chitosan/carrageenan, and they found that the type of polymers used as emulsifiers influenced the release and the topical delivery of the active ingredient in emulsions. Liu et al. [7] showed that whey protein isolate (WPI)–chitosan electrostatic interactions played an important role in the physical property, microstructure and lipid digestion of protein-coated lipid droplets, in the simulated gastrointestinal model, the structures were more stable and the lipid digestion rates were significantly decreased in WPI–chitosan stabilized emulsions compared to WPI-stabilized ones.

Many teams have prepared new types of polymatic emulsifiers using a layer-by-layer (LbL) assembly technique [8–9], their results demonstrated that the mixture of protein and lipids, or protein–polyphenol conjugates and polysaccharides could form stable multilayers at interfaces. The LbL electrostatic deposition is a promising technique, which is usually performed in aqueous medium and does not require harsh conditions [10]. In this approach, a protein usually adsorbs at the droplet surface to form primary emulsion, then the polysaccharide with opposite charges is added to the system and could adsorb to the droplet to produce secondary emulsion [11]. Secondary emulsion is of benefit because it could be prepared with natural food grade ingredients (proteins, lipids, polysaccharides) using simple processing operations (homogenization and mixing), and they could protect droplets from the aggregation or prevent lipid oxidation. Secondary emulsions containing protein/polysaccharide-coated droplets have better stability to environmental stresses than primary ones containing protein-coated droplets [12–13]. On the other hand, it was found that SDS-chitosan-stabilized emulsions were more effective to prevent the formation of citral oxidation product, *p*-cymene, than gum arabic-stabilized ones. These results could be due to the formation of a cationic and thick emulsion droplet interface that could repel pro-oxidative metals [14].

In this study, three milk proteins (lactoferrin (LF), α-lactalbumin (α-La), β-lactoglobulin (β-Lg)) and an anionic polysaccharide (beet pectin (BP)) were applied to form the bilayer emulsion of citral. As one of the most valuable bovine proteins, LF has obtained great interest in food and medicinal researches due to its health benefits [15], and it is an active single-chain glycoprotein with a relatively high isoelectric point (pI ≈ 8.5), and tends to be cationic at low pH [16–17]. α-La is an acidic (pI 4–5), globular, Ca^{2+} binding milk protein [18], due to its nutritional and functional properties, it is widely used in high protein foods, such as infant formula and nutrition bars [19]. β-Lg is a compact globular protein with a pI of about 5.3, which contains 162 amino acid residues with one thiol group and two disulphide bonds. BP is extracted from sugar beet pomace, which was reported to have better surface-activity than high-methoxyl or low-methoxyl pectins [20]. Some studies suggested that the different properties of BP could be due to the presence of acetyl groups (4–5%) [21] and the proteins [22].

To the best of our knowledge, there was no information concerning the physicochemical stability of citral emulsions stabilized by milk proteins and BP. Therefore, the aim of this study was to gain a better understanding of the effect of BP adsorption on the physical and rheological properties of citral emulsions stabilized by milk proteins, moreover, to evaluate the chemical stability of citral in the emulsions during the storage.

2. Materials and methods

2.1. Materials

Citral (the major constituents of the oil were 38.3% neral and 60.5% geranial) was obtained from Symrise AG. (Hozeminden, Germany). Medium chain triglyceride (MCT) was obtained from Lonza Inc. (Allendale, NJ, USA). LF (purity ≥ 97.3%) was purchased from Westland Milk Products (Hokitika, New Zealand). α-La (purity > 90%) and β-Lg (purity > 93.4%) were obtained from Davisco Foods International Inc. (Le Sueur, MN, USA). BP (batch GR93208, Lot 1005–32) was supplied by CP Kelco (Lille Skensved, Denmark). Undecane (purity > 99%) was supplied by Xiya Reagent Co. (Shandong, China). All other chemicals were of analytical grade, unless otherwise stated.

2.2. Solution preparation

LF, α-La, β-Lg and BP (0.7 wt%) were individually dispersed in acetate buffer (10 mM, pH 3.5) and stirred overnight to ensure complete dispersion and dissolution. Sodium azide (0.02 wt%) was added as an antimicrobial agent. Oil phase was made by mixing citral with the carrier oil MCT (1:1, w/w).

2.3. Emulsion preparation

The bilayer emulsions were prepared according to Zhao et al. [23] with some modifications. Primary emulsion was prepared by mixing protein solution (0.7 wt%) with oil phase (10 wt%) at a speed of 10,000 rpm for 3 min by an Ultra-Turrax (T25, IKA, Staufen, Germany). Then the coarse emulsion was further homogenized using a Niro-Soavi Panda two-stage valve homogenizer (Parma, Italy) for three cycles at 60 MPa. Secondary emulsion was prepared by diluting primary emulsions with aqueous BP (0.7 wt%) at a ratio of 1:1 (w/w) to make the final concentration of 0.35 wt% milk protein, 0.35 wt% BP and 5 wt% oil phase. These emulsion systems were stirred in a blender (T25, IKA, Staufen, Germany) at a speed of 10,000 rpm for 3 min, followed by three passes at 60 MPa through a two-stage valve homogenizer (Parma, Italy).

2.4. Zeta-potential measurement

Zeta-potential of the emulsions was determined by measuring the direction and velocity of droplet movement in a well-defined electric field using a Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK). Emulsions were diluted to a final oil droplet concentration of 0.005 wt% with the acetate buffer solution to avoid multiple scattering effects. The data were collected from at least 20 sequential readings per sample after 120 s of equilibration, and the